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Bacterial communities in soil become sensitive to drought under intensive grazing

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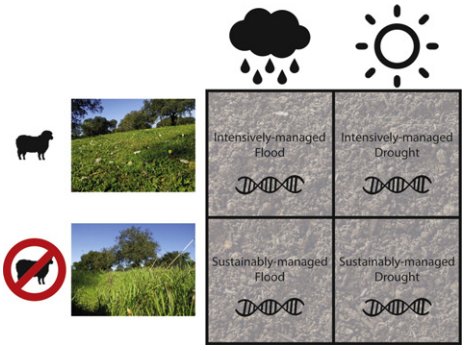
HIGHLIGHTS

- We monitored intensively and sustainably managed soils to month-long drought and flood events.
- Bacterial communities in sustainably managed soils contained less rapidly-growing taxa.
- Only intensively-managed soils were affected by drought.
- Opportunism and stress-tolerance are key traits shaping the bacterial community during recovery.
- The response of several taxa to precipitation depended on land management.

GRAPHICAL ABSTRACT



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ABSTRACT

Increasing climatic and anthropogenic pressures on soil ecosystems are expected to create a global patchwork of disturbance scenarios. Some regions will be strongly impacted by climate change, others by agricultural intensification, and others by both. Soil microbial communities are integral components of terrestrial ecosystems, but their responses to multiple perturbations are poorly understood. Here, we exposed soils from sustainably- or intensively-managed grasslands in an agro-silvo-pastoral oak woodland to month-long intensified drought and flood simulation treatments in a controlled mesocosm setting. We monitored the response of the bacterial communities at the end of one month as well as during the following month of recovery. The communities in sustainably-managed plots under all precipitation regimes were richer and more diverse than those in intensively-managed plots, and contained a lower proportion of rapidly-growing taxa. Soils from both land managements exhibited changes in bacterial community composition in response to flooding, but only intensively-managed soils were affected by drought. The ecologies of bacteria favored by both drought and flood point to both opportunism and stress tolerance as key traits shaping the community following disturbance. Finally, the response of several taxa (i.e. *Chloracidobacteria RB41*, *Janthinobacterium sp.*) to precipitation depended on land management, suggesting that the community itself affected individual disturbance responses. Our findings provide an in-depth view of the complexity of soil bacterial community responses to climatic and anthropogenic pressures in time, and highlight the potential of these stressors to have multiplicative effects on the soil biota.

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1. Introduction

The increased frequency and severity of soil flood and drought events predicted from climate change (Donat et al., 2016) and growing anthropogenic pressures (i.e., agricultural intensification and pollution) are expected to create a patchwork of distribution of environmental changes. The manner in which these stressors affect local communities adds further complexity to the effects of climate change. In soil, bacterial communities are key regulators of nutrient cycles, with the potential to alter global carbon, nitrogen and greenhouse gas budgets (Jeffery et al., 2010). However, the effects of these combined stressors on soil bacterial communities are poorly understood (de Vries and Shade, 2014).

A wide range of studies on the disturbance-responses of bacterial communities generally finds that community functions may be maintained despite disturbance, likely due to the large numbers of bacteria performing the same functions in the extremely diverse soil microbial community. Bacterial community structure measured at the phylogenetic level is more vulnerable, however (Allison and Martiny, 2008). At both phylogenetic and functional levels, disturbance responses can be measured in terms of resistance, or the degree to which a community withstands change in the face of disturbance (Shade et al., 2012) and resilience, the process of reorganization towards the initial state following a disturbance (Hodgson et al., 2015). Importantly, the response of a given bacterial community to disturbance is highly dependent on the type of disturbance, the presence organisms capable of withstanding the disturbance (resistant), as well as on the recovery time (Jurburg et al., 2017a).

Here, we studied the responses of soil bacterial communities underlying sustainably- and intensively-managed Mediterranean agro-silvo-pastoral oak woodlands to month-long flood and drought simulations. Bacterial communities in soils under intensive agricultural management often exhibit different community structures and higher biomass than those under sustainable management (Bissett et al., 2011; Garbeva et al., 2003; Montecchia et al., 2011). These changes might also lead to a reduction in functional potential, given that bacterial communities underlying a harvested forest exhibited a significant, 16% decrease, in biomass decomposition genes even 12 years after harvesting as measured by metagenomics (Cardenas et al., 2015). Here, intensively-managed soil plots were subjected to regular grazing by sheep, as well as agrochemical inputs to improve yield. It is estimated that 60% of the world's agricultural lands are used for grazing (Houghton, 1994), and previous studies have shown that intensive grazing affects the soil bacterial community structure (Ingram et al., 2008; Roux et al., 2008; Klumpp et al., 2009). Sustainably-managed plots were exposed to minimal human interference: grazing was limited to natural fauna, soils received no agrochemical inputs, and endemic vegetation grew freely for over two decades (Table S1 and Fig. S1).

In addition to soil managing strategies, floods and droughts also strain the local bacterial communities by creating physiological challenges, including maintaining osmotic balance and avoiding desiccation (Schimel et al., 2007). At a community level, the potential for altered precipitation patterns to modify soil bacterial community structure has been previously explored (Singh et al., 2010; Wallenstein and Hall, 2012). For example, drying and rewetting may result in long-term changes to bacterial community structure (Fierer et al., 2003; Pesaro et al., 2004). Furthermore, bacterial communities pre-exposed to climate-related perturbations (i.e. changes in precipitation and temperature) were shown to be more resistant to similar stresses in the future than those that did not experience such perturbations (Bérard et al., 2012; Evans and Wallenstein, 2012). In contrast, fungal communities were less affected (Barnard et al., 2013).

The combination of the aforementioned stressors may have multiplicative consequences for soil ecosystems (Paine et al., 1998), and the frequency with which these co-occur is likely to increase (Smith et al., 2016; Donat et al., 2016). However, the response of soil bacterial communities to combinations of stressors is still a significant gap in our knowledge (de Vries and Shade, 2014). A multiplicative effect has been found when stressors present different physiological challenges (Müller et al., 2002; Tobor-Kaplon et al., 2005). For example, studies examining the response of microbiomes in heavy metal-contaminated soils to transient heat stress found that the combination of both disturbances results in decreased substrate utilization capacity relative to controls as well as alterations in community structures, as evaluated by PCR-DGGE (Kuan et al., 2006). In one case, the bacterial community (as evaluated by PCR-DGGE) in an organo-mineral pasture soil exhibited less changes in response to benzene amendment than a mineral arable soil (Girvan et al., 2005). This multiplicative effect may result from the reduction in diversity caused by the initial perturbation, which would result in a reduced number of bacterial populations capable of responding to the second disturbance (Vinebrooke et al., 2004; Jurburg et al., 2017b).

Changes in land management present long-term environmental factors that require the soil community to adapt to new conditions, while flood and drought are transient, allowing the system to return to its pre-disturbance state (Shade et al., 2012). Their combined effect is complex. For example, altered vegetation affects the underlying microbiota's response to drought (de Vries et al., 2012), and soils without agrochemical inputs are better able to retain nutrients during drying-rewetting events than those with fertilizer (Gordon et al., 2008). We hypothesized that sustainably-managed soils would contain more diverse bacterial communities than intensively-managed soils, and that such diverse communities would be more resistant to the precipitation treatments due to a greater level of functional overlap between taxa. Prior exposure to a disturbance may increase a bacterial community's resilience to further exposure, particularly in the case of changes in precipitation (Fierer et al., 2003; Evans and Wallenstein, 2012; Ho et al., 2016). We expected that the intensively-managed, grazed soils would recover from the treatments more rapidly than their sustainably-managed counterparts, as they are regularly exposed to more extreme environmental fluctuations due to a diminished vegetation cover and higher frequency of compaction. We also expected the flood treatment to elicit a more severe response than the drought treatment, as the Mediterranean grassland studied experiences yearly droughts, and its microbiomes should be more adapted to drought regardless of land management.

We thus applied intensified precipitation regimes by selecting precipitation maxima and minima from the last two decades of local (Mediterranean) precipitation data, creating soil moisture conditions that were incrementally stronger than those in previous years. We thus avoided exposing the soil bacterial communities to completely novel ecological challenges, which may elicit more drastic responses (Hawkes and Keitt, 2015). In fact, the gradual intensification of precipitation regimes across the globe is expected to promote gradual community adaptation in the long term (Wallenstein and Hall, 2012). We exposed intact field-collected soil cores from intensively- and sustainably-managed grasslands to month-long intensified drought and flood scenarios while preserving the overlying vegetation, and studied the bacterial communities for an additional month as they recovered.

2. Methods

2.1. Field site

Samples were taken from an agro-silvo-pastoral oak woodland (*Quercus suber* and *Quercus ilex*) located in the Alentejo region of Portugal. The area is located ~290 m above sea level and exhibits a Mediterranean climate, with a mean annual precipitation of between 600 and 1000 mm. Our sampling site consisted of two contiguous plots: a sustainably-managed plot (38°42′20″ N, 8°18′29″ W, pH ~ 5.9) and an adjacent, intensively-managed plot (38°42′14″ N, 8°20′19″ E, pH ~ 5.6). The sustainably-managed plot was characterized by the exclusion of grazing by sheep, and the proliferation of endemic vegetation for >20 years. The intensively-managed plot was exposed to regular

grazing by sheep, active plowing and sowing of palatable herbs for grazing. The soil from both plots was sandy loam. Further information about each plot's nutrient contents and a list of vegetation is available in Tables S1 and S2, and Fig. S1.

2.2. Simulation of intensified precipitation events

In order to simulate intensified month-long rainfall and drought events, we selected the most intensified precipitation events between 1994 and 2013 from regional precipitation records from the Mitra station, in the Évora Geophysics Center database (http://www.cge.uevora.pt/pt/component/cge_bd/?cge_bd_e_first=mit). The drought regime was developed by averaging the mean daily precipitation for summer months (June, July, and August, 0.33 mm/day). The flood regime was developed by averaging all high-precipitation events from the 20 year record, defined as three or more days of >10 mm of precipitation per day within a seven-day period (20 mm/day). The control rain regime was the mean annual precipitation for the 20 years of daily data available (~1.76 mm/day). These were applied to cores from each management regime, in triplicate.

2.3. Mesocosm experiment

We employed Terrestrial Model Ecosystems, in which a large cylinder of soil is extracted from the land and moved to a laboratory setting, preserving overlying vegetation and soil structure (Rombke et al., 2004; van Elsas et al., 1986). Eighteen intact soil cores were extracted in March 2014 using a soil core extractor, which contained a high-density polyethylene tube that also served as an encasement for the cores. Cores measured 40 cm in length and 16.5 cm in diameter. Nine cores were extracted from each of the sustainably- and intensively-managed plots. Gauze separated the cores from the bottom of the cylinder, which was fitted with PVC pipe, allowing soil cores to drain. Cores were transported to temperature-controlled carts, which maintained a vertical temperature gradient similar to that expected along a vertical gradient in soil (from 14 °C at the lowest point to 21 °C at the surface). The carts were maintained in a temperature-controlled chamber at 21 °C, and were partially isolated from each other by clear plastic enclosures at the soil surface. All cores were kept at control precipitation conditions (~1.76 mm/day) for 20 days, and were then subjected to treatment or control regimes for 28 days: 6 cores (3 from sustainably-managed and 3 from intensively-managed plots) were subjected to the drought regime (0.33 mm/day), 6 were subjected to the flood regime (20 mm/day), and 6 were maintained under control precipitation (~1.76 mm/day), resulting in triplicate cores for each management × treatment combination. Finally, all cores were returned to control precipitation regimes (~1.76 mm/day) for 26 days of recovery. Throughout the experiment, grasses in intensively-managed mesocosms were partially removed by plucking every ~10 days to simulate grazing. Watering took place three times per week, using artificial rain water prepared according to Velthorst (1993), and soil moisture content was monitored by inserting soil probes (Decagon devices Inc., Pullman, WA) into one core per management × treatment combination. Soil moisture data is available in Fig. S2.

Soil samples were obtained from all cores on the 30th and 32nd day of the intensified precipitation treatment to study resistance, as well as on the 5th, 12th, and 26th days of recovery. At each sampling time, 3 small cores (diameter 2 cm; length 15 cm) were collected from each core at a random location, avoiding the moisture probe and the edges of the core. The three soil cores from each mesocosm were pooled, homogenized and sieved through a ~ 2 mm sieve prior to analyses. Holes resulting from sampling were filled with PVC pipes to reduce disturbances in the structure of the soils.

For each time point and each core, DNA was extracted from 0.5 g of fresh soil using the MoBio PowerSoil DNA Extraction Kit (MoBio Laboratories, Carlsbad, CA, U.S.A.) following the manufacturer's instructions, with three additional rounds of bead-beating for 30 s using a Tissue Lyser LT equipment (Qiagen, Hilden, Germany). The concentration and band size of the resulting products were quantified by electrophoresis using a 0.8% agarose gel with a SmartLadder (Eurogentec, Liege, Belgium). Two field samples—one from the sustainably-managed and one from the intensively-managed plots—were extracted during the extraction of the mesocosm cores, and were processed using the same procedure.

2.4. 16S rRNA copy number quantification

The number of 16S rRNA gene copies in the total community DNA were used to estimate the number of bacteria throughout the experiment. Copy number quantification was performed using an ABI PRISM 7300 Cycler (Applied Biosystems, Germany) as previously described (Pereira e Silva et al., 2012) by targeting the 264-bp V5–V6 region of the 16S rRNA gene (primers 16SFP/16SRP (Bach et al., 2002)). A standard curve spanning six orders of magnitude (10^2–10^8) was generated using linearized plasmids containing a 16S rRNA gene cloned from a *Burkholderia* species. Amplification efficiency (E) was calculated according to the equation, $E = (10^{-1/\text{slope}} - 1)$. For both runs, $E = 100 \pm 10\%$. All data are shown as log gene copy number g^{-1} fresh soil.

2.5. 16S rRNA gene amplicon sequencing and analyses

16S rRNA gene amplicon sequences were used to monitor bacterial community composition. Sequencing was performed using an Illumina MiSeq system at Argonne National Laboratory (Argonne, IL, U.S.A) as previously described (Caporaso et al., 2012). The raw sequences were analyzed using QIIME 1.9.0 (Caporaso et al., 2010). Briefly, forward and reverse paired-end sequences were joined using the join_paired_ends.py command, and FastA headers were made QIIME-compatible with the split_libraries_fastq.py command. Operational taxonomic unit (OTU) picking was performed with the pick_open_reference_otus.py command, using the default uclust method and the Greengenes database, with the suppression of step 4 of the command.

Further analyses were performed in R 3.2.3 (R Core Team, 2014) using the *Phyloseq* (McMurdie and Holmes, 2013) package. Singletons were removed and all samples were rarefied to 29,300 reads per sample using rarefy_even_depth from the Phyloseq Package (seed.number = 266,315). Sequencing and processing led to the loss of five samples: two from intensively-managed controls from the 32nd day of treatment and the 12th day of recovery, one from intensively-managed soils exposed to the flood treatment on the 12th day of recovery, and two samples from the sustainably-managed controls on the 12th day of recovery. The final dataset contained 2,549,100 reads distributed into 87 samples and 13,402 OTUs.

2.6. Statistical analyses

α-Diversity was calculated using the Shannon-Wiener diversity index and Pielou's evenness, in the R environment, using the *vegan* package (Oksanen et al., 2007). Differences between treatments were evaluated with Kruskal-Wallis tests for α-diversity as well as for the total 16S rRNA gene copy counts. Pairwise differences were evaluated using a post-hoc Tukey test after Nemeyi from package *PMCMR* (Pohlert, 2014).

To characterize the bacteriomes of both plots in the absence of precipitation extremes, we included only samples from control mesocosms for the sustainably-and intensively-managed plots. To evaluate immediate changes in community composition in response to the intensified precipitation treatments we created ternary plots of taxa with average abundances >0.1% in samples from the 30th and 32nd day of treatment for each management. The percentage of total reads for each OTU found in each treatment was plotted using package *ggtern* (Hamilton, 2015). A

principal coordinates analysis (PCoA) of Bray-Curtis distances was used to visualize the relative differences between management, experimental treatments, sampling time (merged into two categories, Treatment or Recovery phases), and between core and field samples. PERMANOVAs of Bray-Curtis distances between samples were performed using *RVAideMemoire* (Hervé, 2012). Principal response curves (PRCs) were used to follow bacterial community recovery (Van den Brink and Ter Braak, 1999). We selected 79 taxa with species scores > |2| in a PRC including all managements, only sustainably-managed or only intensively-managed soils for further analyses. For the heat map, these taxa were centered and scaled, Euclidean distances were calculated and taxa were clustered using Ward's method.

3. Results

3.1. Bacterial abundance and α-diversity

Total bacteria, quantified in terms of numbers of copies of bacterial 16S rRNA genes, exhibited no significant effect of either soil management, treatment or whether the samples were taken during treatment or recovery (Kruskal-Wallis χ^2 test, $p > 0.4$ for all comparisons, Fig. S3). Thus, using the total bacterial community DNA, similar sizes of bacterial biomass were estimated across all samples, regardless of treatment or management.

In contrast, the α-diversity values differed between the soils from the different treatments and management types (Fig. 1). For all samples, sustainably-managed soils were richer than intensively-managed soils: the mean of Shannon's H was 6.599 and 6.507 respectively (1-tailed *t*-test, $p = 0.006$). Sustainably-managed soils were also more even than intensively-managed soils, with a mean of Pielou's evenness of 0.812 and 0.804, respectively (1-tailed *t*-test, $p = 0.022$). Kruskal-Wallis χ^2 tests also revealed significant effects of *management* × *treatment* on richness ($\chi^2 = 14.687$, $p = 0.040$) and evenness ($\chi^2 = 16.582$, $p = 0.020$), as well as of *management* × *time* × *treatment* on richness ($\chi^2 = 16.102$, $p = 0.003$) and evenness ($\chi^2 = 18.183$, $p = 0.001$), suggesting that the communities changed in response to the treatments.

3.2. Resistance of the bacterial community to treatment effects

To characterize the (control) bacterial communities in both management regimes, we analyzed the soil samples collected in the control mesocosms from all time points in each management type. The communities in the soils from both plots were similar at the higher taxonomic levels, and dominated by members of the Proteobacteria, Acidobacteria, Verrucomicrobia, Actinobacteria and Bacteroidetes. One-tailed *t*-tests between cores from sustainably- and intensively-managed soils revealed a significantly higher proportion of Actinobacteria, Verrucomicrobia, Firmicutes, Nitrospira and Planctomycetes in sustainably-managed cores, and a significantly higher proportion of Proteobacteria (mostly α-Proteobacteria), Bacteroidetes, Gemmatimonadetes and Cyanobacteria in intensively-managed cores (Fig. S4, $p < 0.01$ for cases with significant differences).

In order to quantify the immediate effect of the precipitation treatments on the communities, we compared control samples with those exposed to intensified precipitation regimes from days 30 and 32 of treatment (Fig. 2). The analysis showed that the effects of the drought and flood treatments on the communities were largely dependent on land management. Sustainably-managed soils exposed to flood exhibited increases in the relative abundances of *Koribacter sp.* (Acidobacteria), *Pedobacter* sp. and several unclassified members of the Chitinophagaceae (Bacteroidetes), as well as several proteobacterial OTUs including *Acinetobacter sp.*, *Janthinobacterium* sp. and *Luteibacter sp.*. No major changes were observed from exposure to the drought treatment.

On the other hand, the intensively-managed soils exposed to flood exhibited increases in the relative abundances of *Sphingobacterium sp.* (Bacteroidetes) as well as proteobacterial OTUs assigned to Comamonadaceae (Proteobacteria) and a single OTU classified as a *Janthinobacterium sp.*. Notably, intensively-managed soils exposed to drought exhibited increases in the relative abundances of *Streptomyces* sp. and *Glycomyces sp.* (Actinobacteria); *Acinetobacter* sp. and an *unclassified Xanthomonadaceae* OTU (Proteobacteria); *Sphingobacterium* sp. and *Chitinophaga sp.* (Bacteroidetes).

3.3. Bacterial community recovery

Clear and significant differences were observed between the communities sampled during *treatment* and *recovery* for each mesocosm, suggesting that the community underwent post-disturbance reorganization (Fig. 3). A PERMANOVA of the Bray-Curtis distances of all bacteriomes revealed significant differences between the community structures from intensively- and sustainably-managed plots exposed to flooding during recovery. Moreover, the communities in intensively-managed soils subjected to drought were different from those in the controls ($p < 0.01$ for all pairwise comparisons), whereas no such differences were observed for the drought treatments in the sustainably-managed soils, further supporting that the bacterial communities

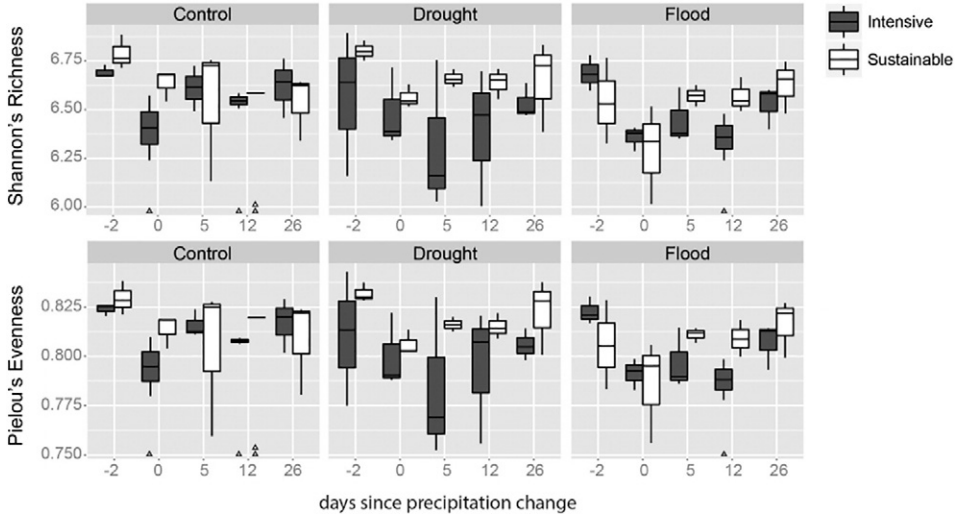

Fig. 1. Changes in bacterial α-diversity over time, measured as Shannon's richness (top) and Pielou's evenness (bottom), grouped by precipitation treatment (control, drought, flood) and management type (intensively versus sustainably managed soils). Error bars represent standard error. Triangles indicate a sample lost during processing; $n = 3$ otherwise.

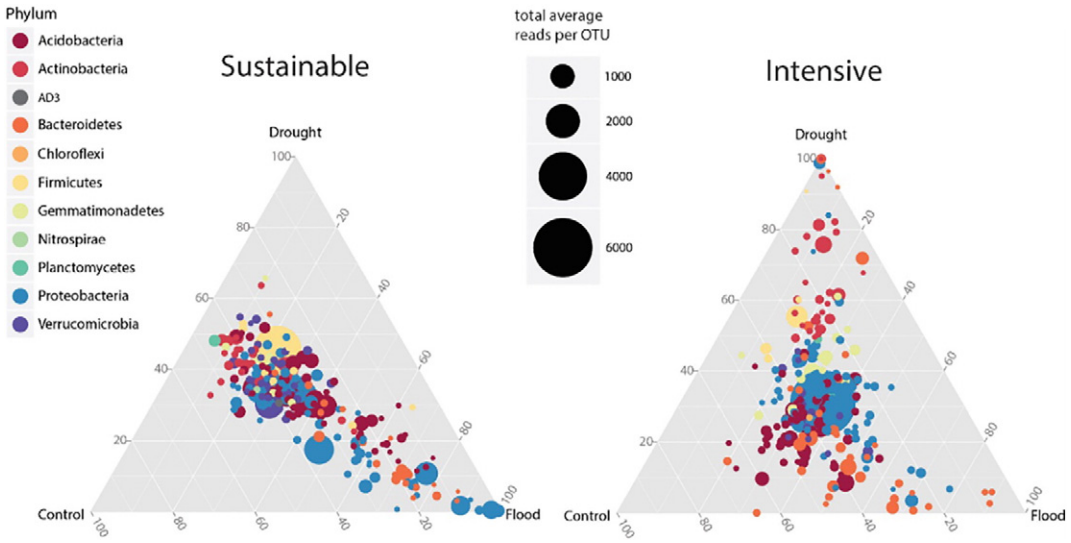

Fig. 2. Ternary plot of bacterial communities from the end of the intensified precipitation treatments (days 30 and 32 of treatment). The most abundant bacterial operational taxonomic units (OTUs) representing 50% of the community were selected. The position of each point is determined by the percentage of reads of that OTU which were found in each treatment. Circle size is proportional to the average abundance of an OTU in the three precipitation treatments from the same management type. Color indicates the OTU's phylum.

in intensively-managed soils were vulnerable to drought, but those from sustainably-managed soils were not.

We performed a principal response curve (PRC), which showed a clear separation between soil management types, but the effect of precipitation treatments over time was less apparent (Fig. S5). The loss of samples during processing precluded the confirmation of the significance of each treatment relative to controls. To further explore the community dynamics, we selected OTUs with species scores >2 or smaller than −2 in the PRC for inspection, as these are expected to play a more substantial role in the temporal patterns observed in community composition (Fig. 4). These 79 OTUs made up 34% of the communities on average, and could be grouped into two main clusters: (1) OTUs which were predominantly present in sustainably-managed soils and (2) those which were mostly present in intensively-managed soils. For example, 7 OTUs of classified as different strains of *Chthoniobacter DA101* were most abundant in sustainably-managed soils under control conditions, (together, 8% of the community on average). They decreased under flood and drought conditions (5% and 6% of the community on average, respectively), but were at lower relative abundances in intensively-managed soils under all conditions (2% of the community on average, Fig. 4). In contrast, 5 OTUs classified as different strains of *Kaistobacter* sp. exhibited highest relative abundances in intensively-managed soils exposed to flooding and drought, particularly on the 5th day of recovery (together, 14% of the community on average for both treatments), slightly lower relative abundances in control soils (9.5% of the community on average), and much lower ones in sustainably-managed soils (2% of the community on average for all treatments, Fig. 4). Notably, 13 OTUs assigned to different strains of *Koribacter* or *Koribacteraceae* exhibited different patterns. Those which were most abundant in intensively-managed soils were negatively affected by drought. For example, one OTU exhibited an average relative abundance of 0.5% in intensively-managed control soils, 0.7% in soils exposed to flood, and 0.2% in soils exposed to drought. Five OTUs assigned to *Sphingobacteriaceae* and *Pedobacter* sp. were more abundant in intensively-managed control soils than in sustainably-managed soils (1.5% and 0.5% of the community in total, respectively), but increased in sustainably-managed soils exposed to flooding (1.5% of the community in total), particularly during the flooding, and decreased during recovery back to control levels. In both soil management types, several of these OTUs exhibited highest relative abundances during treatment, and rapidly decreased during recovery. In contrast, three members of the *Burkholderiales* (*Janthinobacterium sp.*, *Ralstonia sp.*, and an unclassified OTU) had similar relative abundances in both soil management types (0.3% of the community in total), but increased to a greater degree in sustainably-managed soils when exposed to flooding (4% and 0.8% of the community, respectively). Finally, the response of some lowly-

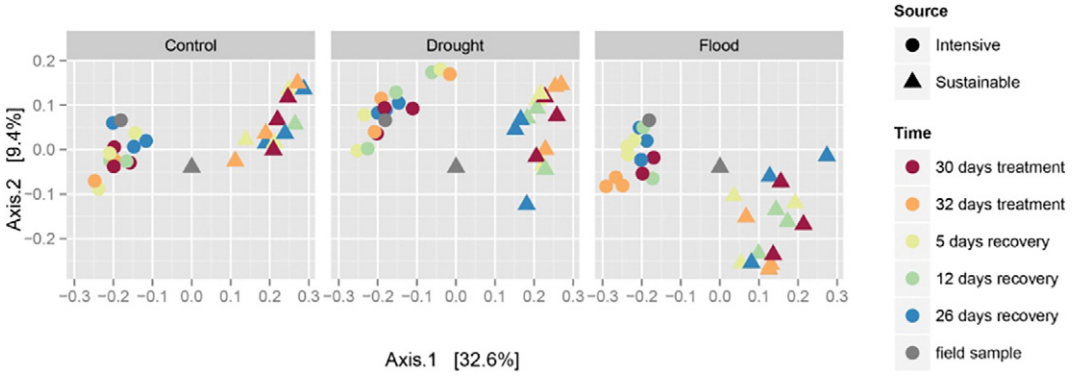

Fig. 3. Bacterial community composition over time. A single Bray-Curtis PCoA, grouped by treatment. PERMANOVAs showed a highly significant difference between intensively- and sustainably-managed soils for all treatments and times ($p = 0.001$), and a highly significant effect of management * treatment ($p = 0.0011$), except for sustainably-managed soils exposed to drought or control conditions ($p = 0.089$).

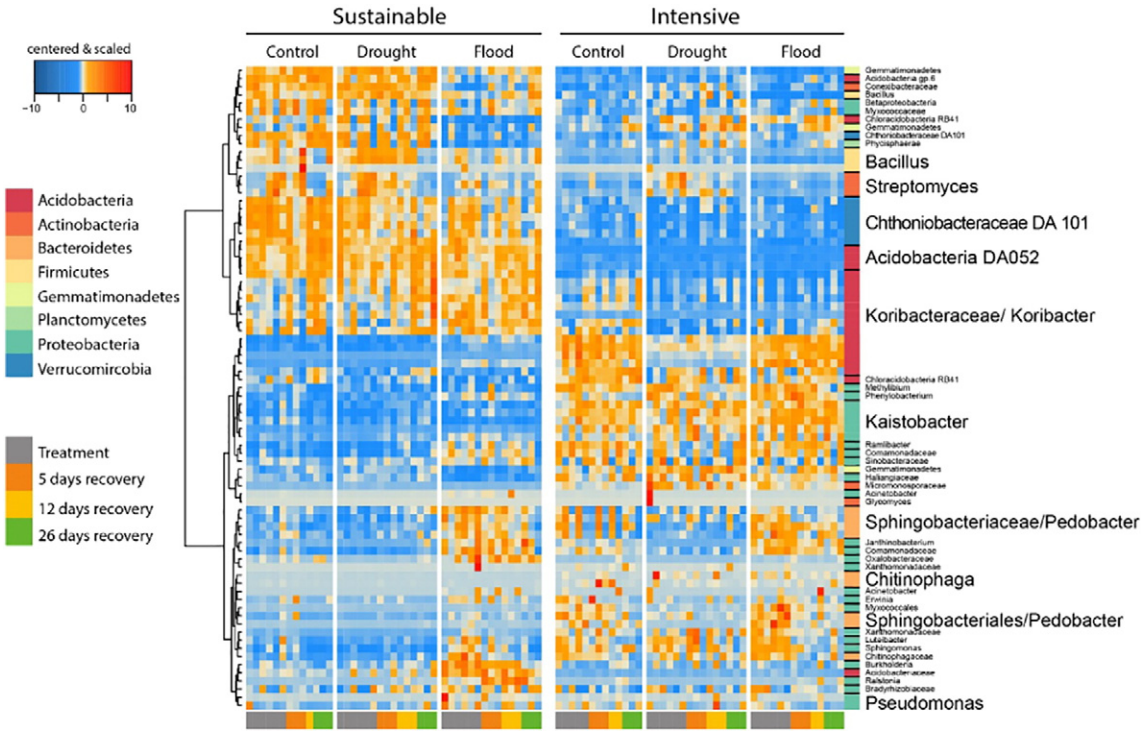

Fig. 4. Heatmap of bacterial taxa driving changes in community composition during the experiment. Taxa with species scores > |2| in one of three principle response curves (all treatments and managements, all treatments in intensively-managed soil, or all treatments in sustainably-managed soil) were included. Taxon abundances were centered and scaled per operational taxonomic unit. Clustering is based on Ward's method, using Euclidean distances. These taxa represent 34% of the total community on average. The color bar on the bottom indicates sampling time, and *Treatment* includes samples from the 30th and 32nd days of precipitation treatments. The color bar on the right indicates the phylum of each OTU. Different OTUs are separated by a black line.

abundant taxa (<0.5% of the community) to flood depended on the management type. For example, an *unclassified Acidobacteria OTU* decreased from 0.6% to 0.3% in the sustainably-managed flood treatment but remained low (0.2%) in all intensively-managed mesocosms. In another example, an OTU matching *Chloracidobacteria RB41*decreased relative to the controls from 0.3% to <0.1% of the community in the flood treatment for sustainably-managed mesocosms, but significantly increased relative to the controls from 0.2% to 0.4% of the community in flood and drought treatments for the intensively-managed mesocosms (Fig. 4).

4. Discussion

Given the increase in anthropogenic pressures worldwide and the forecasted intensification of precipitation patterns, it is crucial to understand whether the resilience of soil ecosystems under stress is impaired. Our findings show that, in an agro-silvo-pastoral oak woodland ecosystem with differential grazing intensity, the response of soil bacterial communities to intensified precipitation events clearly depends on the land management.

Employing mesocosms allowed us to preserve the overlying vegetation and soil density. While this likely increased the potential for ecological feedbacks and the complexity of interactions as well as the variability throughout the experiment, it also allowed to observe soil communities in a more realistic setting than in culture or in microcosms, targeting surface and subsurface bacterial communities. Differences the vegetation composition and density between land management types may explain the higher moisture content in the sustainably-managed soils under all treatments, as these had a thicker cover (Fig. S1). As has been documented in other studies (Fierer et al., 2003), this vegetation may have buffered the soils against decreases in precipitation, and its absence may have increased the exposure of bacterial communities in intensively-managed soils to dry conditions in the drought treatment. It is also likely that the differing composition of vegetation cover (Table S2) contributed to shaping the microbiomes throughout the experiment, as has been previously reported (Classen et al., 2015). While we did not explicitly sample rhizosphere microbiomes, it is likely that these were strongly affected by land management (Pérez-Jaramillo et al., 2016), and most likely responded both directly to changes in precipitation and indirectly to changes in vegetation cover (Berard et al., 2015), as the latter also responded to the vegetation treatments. Despite controlled and regular inputs of rainwater, moisture in the intensively-managed soils increased after the cessation of the flood treatment (Fig. S2), and responded more rapidly to changes in the precipitation regimes. We suspect the latter to be an effect of the higher compaction in these soils, which by reducing pore size and connectivity, exposed the communities to more fluctuations (Hartmann et al., 2014).

The higher rate of fluctuation in moisture in the intensively-managed soils may partially explain the differences found in the original bacterial community compositions of the two management types. In the intensively-managed mesocosms, the larger proportion of *α-Proteobacteria* and *Bacteroidetes*—which contain many opportunistic phyla with potentially fast growth responses (Fierer et al., 2007)—points to an effect of the larger fluctuations of moisture conditions favoring rapid growth and adaptation in these soils. The higher proportion of phyla with many slow-growing or sensitive taxa (*Actinobacteria*, *Verrucomicrobia*, *Firmicutes*, *Nitrospira* and *Planctomycetes*; Buckley et al., 2006; Attard et al., 2010; Fierer, 2015) in sustainably-managed soils supports the notion that these were exposed to less environmental change, which favored specialists.

Only communities from intensively-managed mesocosms exhibited changes in response to drought. The lowered response to the drought treatment relative to the flood treatment agrees with the characteristically dry Mediterranean climate, which would have resulted in the adaptation of soil microbes to some degree of drought, as previously reported (Barnard et al., 2013, 2015). Increases in the relative abundances of *Streptomyces* and other *Actinobacteria* relative to the controls

were observed. These taxa flourish upon changes of moisture in soil (Barnard et al., 2013), particularly drought (Williams et al., 1972; Acosta-Martiez et al., 2014; Berard et al., 2015), indicating that the drought favored drought-tolerant taxa. Drought also resulted in an increase of rapid-growing, opportunistic taxa with the ability to metabolize complex hydrocarbons such as *Acinetobacter* sp. (Doughari et al., 2011), *Sphingobacterium sp*., and *Chitinophaga sp*. (Normand et al., 2014). This suggests that a lack of connectivity between soil pores may have resulted in starvation, favoring soil bacteria able to degrade recalcitrant and less palatable carbohydrates for energy.

In sustainably-managed soil mesocosms, no significant changes were observed from the drought relative to the controls, which may be because the taxa favored by the drought treatment in intensively-managed soils were already present in high proportions in sustainably-managed soils prior to treatment. In this sense, the higher richness and evenness found in the latter may have acted as an insurance against environmental change (Yachi and Loreau, 1999; Wittebolle et al., 2009), as sustainably-managed soils needed to change less to adapt to the drought conditions. In addition, the more diverse bacteriomes from the sustainably-managed soil plots contained a higher proportion of disturbance-tolerant taxa. Thus, the system had less open niche space during and after the drought, and probably witnessed weaker successional dynamics for the colonization of these niches. In the less diverse, intensively-managed soils, adapting to drought required more drastic changes in the community, suggesting that the tolerances of individual community members are key determinants of bacterial community disturbance responses (Lennon et al., 2012; Awasthi et al., 2014). The increased vulnerability of intensively-managed soils to drought, however, shows that the alteration of soil microbial communities by human activities—here intensive grazing—can affect the bacterial community's response to climate change. Whether these altered responses have an effect on the provision of ecosystem services by soil bacteria is still a matter of debate (McGuire and Treseder, 2010; Nemergut et al., 2014), and deserves further study.

A strong effect of flooding was observed for soil bacteriomes from both management types. Communities from sustainably-managed soils became more similar to those of intensively-managed soils during and after flooding, exhibiting increases for several members of *Burkholderiales* (i.e., *Burkholderia sp*., *Bradyrhizobium sp*., *Ramlibacter sp*.). In intensively-managed soils, these taxa were already present in higher abundances prior to flooding. In soils from both management types, copiotrophs such as Proteobacteria and *Bacteroidetes* (Fierer et al., 2007; Schimel et al., 2007) increased in relative abundance. This may have resulted from the redistribution of resources within the soil matrix, favoring bacteria able to consume them rapidly (Wilson et al., 2011; Placella et al., 2012). We also detected an increase in the relative abundance of flood-resistant taxa such as *Janthinobacterium sp*., which belongs to a genus that contains efficient genes for osmotic stress tolerance (Shoemaker et al., 2015). It is important to note, however, that cells inactivated by drought with intact DNA may have been detected as present by the molecular methods employed.

Previous studies have shown that individual stress tolerance ranges (Lennon et al., 2012; Evans and Wallenstein, 2014) modulate community responses to changes in soil moisture. The repeated sampling of the soil bacterial communities in our experiment showed that resource use and growth—particularly opportunists and r-strategists—are also favored by changes in precipitation and land management (de Vries and Shade, 2014). We have previously shown that both strategies are important during secondary succession in soil bacterial communities (Jurburg et al., 2017a).

Most notably, we found that the response of several lowly abundant taxa to flooding was dependent on land management type. This aligns with a recent study which shows that the ecological strategies of several bacteria depend on the soil's disturbance history, in their case multiple drying-rewetting cycles (Evans and Wallenstein, 2014). The authors suggest that this is a result of ecological filtering of traits, evolution, or horizontal gene transfer. We further propose that community composition itself may play a role in selecting and modulating physiological traits. For example, while the abundance of members of the genus *Burkholderia* is negatively related to soil pH, a wider range of *Burkholderia* strains are able to grow at high pH (~11). Thus the genus is generally acidotolerant, not acidophilic, and is outcompeted by better adapted bacteria at higher pH. Likewise, the different responses observed for specific taxa from different land management types exposed to flooding in our experiment may be a result of altered competitive dynamics. This also aligns with the finding that a soil bacterial community's propensity to invasion is determined by resource availability and competitive dynamics (van Elsas et al., 2012; Fiegna et al., 2015; Mallon et al., 2015).

Our findings thus highlight the complexity of soil bacterial community responses to real-world disturbances on multiple levels. Firstly, it is crucial to acknowledge the multiplicative potential of real-world disturbances. In our experiment, grazing decreased vegetation cover and increased soil compaction, which in turn likely increased the vulnerability of altered precipitation patterns by accelerating the rate of drying and flooding. On the other hand, the higher water holding capacity and organic matter content in intensively-managed soils may have acted as a buffer to intensified precipitation changes (Table S1). The responses of other members of the soil biota, including fungi, archaea and metazoans, although not included in this study, were likely affected as well, although the responses might be different. For example, fungal responses to drought are often more pronounced than those of bacteria in soil (Acosta-Martiez et al., 2014). Secondly, the effects of each combination of disturbances is unique: the higher proportion of opportunists in intensively-managed plots decreased their vulnerability to flooding, but the higher diversity and evenness in sustainably-managed plots decreased their vulnerability to drought. Thirdly, the response of individuals is variable, and may depend on factors such as the combination of disturbances, the soil's history, and competitive dynamics within the community. This last finding adds a further complication to determining the outcome of soil disturbance on the community, as the responses may be dependent on the bacterial community structure at the time of perturbation.

Climate change is expected to increase the frequency and magnitude of both flood and drought events, and our results should be considered in conjunction. The bacteriomes of sustainably-managed soils were not affected by drought, but begun to resemble those of intensively-managed soils after flooding. Further research is necessary to determine whether multiple drying-rewetting cycles will make the bacterial communities of soils under different management regimes more similar. The increased response of intensively-managed soils to drought suggests that there are thresholds to community tolerance, which have been previously discussed within the context of laboratory-based, extreme disturbances (i.e., diluting soil bacterial communities at increasing frequencies Kim et al., 2013). Our findings show that that there may be a community-level cost associated with withstanding routine disturbances (the 'cost of tolerance', Berard et al., 2015), which takes a toll on the community's resilience to other perturbations. Extant, routine disturbances, in conjunction with climate change, have the potential to cause real losses in soil bacterial community resilience.

Soil resilience constitutes a fundamental component of soil quality (Seybold et al., 1999). From this perspective, the increased sensitivity of intensively-managed soils to drought signifies a loss of soil quality. A recent theoretical model showed that the sensitivity of soil bacterial communities to environmental change is dependent on whether the soils were subjected to disturbances of similar magnitudes in the past (Hawkes and Keitt, 2015). On the other hand, microcosm experiments showed that soils exposed to increasing frequencies of re-inoculation (every 7–56 days) into sterile soil collapsed at the highest frequency, becoming completely stochastic (Kim et al., 2013). The gradual intensification of disturbances expected from climate change may thus allow soil bacterial communities to adapt. However the increasing frequency

of extreme events eventually may lead to a catastrophic shift, in which the community drastically changes to a new conformation (Scheffer et al., 2001). Another challenge to understanding the effects of climate change is the shifting duration of intensified precipitation events. In our experiment, we selected a treatment duration of 28 days, and it is likely that the observed patterns were highly dependent on this duration (Berard et al., 2015). A longer drought might have resulted in further effects on the soil bacteriomes, if plant mortality increased (Turner and Dale, 1998). Conversely, a longer flood period might have resulted in the selection of anaerobic bacteria, as is, for instance, common in rice paddies (Lee et al., 2015). We tested the effect of two classes of environmental pressures: management and precipitation. However, climate change is expected to result in more extreme fluctuations in temperature as well (Pachauri et al., 2014). The interaction between temperature and precipitation on soil bacterial communities, while often studied, is often not partitioned into the separate effects of each (Berard et al., 2015). The potential interactive effect of different perturbations on soil bacterial communities requires urgent and further study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2017.10.012.

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